

WHAT IS CLAIMED:

1. A thermostable ligase having 100 fold higher fidelity than T4
ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when
5 sealing a ligation junction between a pair of oligonucleotide probes hybridized to a
target sequence where there is a mismatch with the oligonucleotide probe having its
3' end abutting the ligation junction at the base immediately adjacent the ligation
junction.

10 2. A thermostable ligase according to claim 1, wherein said
thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity
than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between
a pair of oligonucleotide probes hybridized to a target sequence where there is a
mismatch with the oligonucleotide probe having its 3' end abutting the ligation
15 junction at the base penultimate to the ligation junction.

20 3. A thermostable ligase according to claim 2, wherein, in the
presence of a Mn²⁺ cofactor, said thermostable ligase has a 12 fold higher fidelity than
wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a
pair of oligonucleotide probes hybridized to a target sequence where there is a
mismatch with the oligonucleotide probe having its 3' end abutting the ligation
junction at the base immediately adjacent to the ligation junction.

25 4. A thermostable ligase according to claim 3, wherein the
thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif
where X is any amino acid.

30 5. A thermostable ligase according to claim 1, wherein, in the
presence of a Mn²⁺ cofactor, said thermostable ligase has a 12 fold higher fidelity than
wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a
pair of oligonucleotide probes hybridized to a target sequence where there is a

mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

6. A thermostable ligase according to claim 5, wherein the
5 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif
where X is any amino acid.

7. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif
10 where X is any amino acid.

8. A thermostable ligase according to claim 1, wherein the
thermostable ligase has a molecular weight of 78 to 81 kDa determined by SDS-
PAGE.

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9. A thermostable ligase according to claim 1, wherein the
thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

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10. A thermostable ligase having 50 fold higher fidelity than T4
ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when
sealing a ligation junction between a pair of oligonucleotide probes hybridized to a
target sequence where there is a mismatch with the oligonucleotide probe having its
3' end abutting the ligation junction at the base penultimate to the ligation junction.

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11. A thermostable ligase according to claim 10, wherein, in the
presence of a Mn²⁺ cofactor, said thermostable ligase has a 12 fold higher fidelity than
wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a
pair of oligonucleotide probes hybridized to a target sequence where there is a
mismatch with the oligonucleotide probe having its 3' end abutting the ligation
30 junction at the base immediately adjacent to the ligation junction.

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12. A thermostable ligase according to claim 11, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

5 13. A thermostable ligase according to claim 10, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

10 14. A thermostable ligase having an arginine adjacent its active KXDG motif where X is any amino acid.

15. A thermostable ligase having, in the presence of a Mn²⁺ cofactor, a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

20 16. An isolated DNA molecule encoding a thermostable ligase, wherein the thermostable ligase has a 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.

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17. An isolated DNA molecule according to claim 16, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

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